Comparison of the Falling Drop Method to Existing Hemoglobin Methods

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What is Anemia?

- Definition
  - Reduced oxygen carrying capacity of the blood

- Causes
  - Nutritional deficiency (vitamins [B12/folate], iron)
  - Increased utilization of nutrients (infections, pregnancy, growth)
  - Inherited conditions (thalassemia, hemoglobinopathies, enzyme deficiencies, membrane defects, porphyrias)
  - Autoimmune hemolytic anemias (ITP - IgG, IgM)
  - Somatic mutations
    - Aplastic Anemia, Congenital Dyserythropoietic Anemia, Paroxysmal Nocturnal Hemoglobinuria
  - Malignancies (leukemias, metastatic malignancies)

Symptoms of Anemia

- Mild Anemia
  - Weakness, fatigue, headache, pallor

- Moderate Anemia
  - Dyspnea, tachycardia, dizziness, irritability

- Severe Anemia
  - Incapacitating fatigue
  - Cognitive deficits
  - Cardiac defects (MI, CHF)
  - Low birth weight
  - Preterm birth
  - Risk of maternal or neonatal mortality

Prevalence of Anemia Worldwide

- Anemia affects 25% of the world’s population
- Underdeveloped countries are more affected
  - All deaths from Iron Deficiency Anemia worldwide
    - 1.4% in North America
    - 71% in Africa and parts of Asia
  - Link between anemia and poverty
  - Nutritional deficiencies
  - Increase in parasitic and bacterial infections
  - Blending and increased utilization
  - Haiti is the poorest country in the Northern and Western hemispheres
    - In 2017, Haiti was ranked the 17th poorest country in the world

How is Anemia Measured

- RBC
  - Automated CBC
  - Manual RBC count

- Hematocrit
  - Automated CBC (calculated from RBC and Hb)

- Hemoglobin
  - Automated CBC (spectrophotometric)
  - POC instruments

Limitations to Modern Hb Measurement in Haiti

- Cost
  - Instrument
  - Reagents
  - QC
  - Electricity

- Stable and reliable electricity
  - CBC Instruments
  - Climate control
  - Refrigeration

- Adequate training of lab staff
- Instrument maintenance and repair
Problem

- Anemia is prevalent worldwide
- More prevalent in underdeveloped countries
- Modern methods are expensive, complex, electricity dependent
- Some Haitian labs do not have any electricity for even microhematocrit
- Sickle Confirm requires a Hb measurement to perform
- Need Hb method that is cheap, fast, easy and electricity independent
- Falling Drop Method

Falling Drop Method

- Principle
  - The rate of descent of a drop of blood down a column through a density gradient is proportional to the hemoglobin content in the drop of blood
- Variables to consider
  - Column size (length and diameter)
  - Vertical positioning of column
  - Density gradient solution (inert and dynamic)
  - Blood drop delivery system (reproducible)
  - Blood drop integrity
    - Anticoagulant used in blood collection (oxalate, citrate, EDTA, heparin)
  - Linear relationship between descent time and Hb content
  - Accuracy
  - Reproducibility
  - Reagent stability

Materials and Methods

- Samples
  - 20 blood samples from healthy, IRB consented, subjects
    - One EDTA tube for all Hb testing except the falling drop method
    - One-potassium oxalate/sodium fluoride for the falling drop method
  - Each sample was diluted 1:2 in autologous plasma for a total of 40 samples
- Falling Drop Set-up
  - Six-61cm glass columns with 10mm inner-bore diameter
  - Start/stop markings at 49.5cm apart
  - Modified, universal ESR rack to hold tubes
  - Modified pipette tips at mouth of column to stabilize blood drop delivery
- Density Solution
  - 4.58% solution of copper sulfate pentahydrate with specific gravity of 1.015

Materials and Methods

- Falling Drop Method
  - A 44uL drop of blood collected in potassium oxalate/sodium fluoride is applied to the surface of the copper sulfate in the column through a modified pipette tip to stabilize drop delivery
  - Descent time is measured between the markings (49.5cm)
  - Six trials per blood sample tested is applied to the column
  - Average of six trials is compared to the standard curve to determine Hb level
- Correlation of Falling Drop to Other Methods
  - Falling Drop method was correlated to:
    - Sysmex KX-21N, Hemocue 201+, Mission Plus Hb, and WHO Visual
  - Descriptive statistics: Mean and standard deviation
  - Inferential statistics: Correlation Coefficient (r) & Coefficient of Determination ($r^2$)

Hemoglobin Methods

Sysmex KX-21N  Hemocue 201+  Mission Plus  WHO Visual
## Correlation of Sysmex to Other Methods

<table>
<thead>
<tr>
<th>Method</th>
<th>Mean Hb (g/dL)</th>
<th>Standard Deviation (g/dL)</th>
<th>Sysmex vs Other Methods</th>
<th>Correlation Coefficient (r)</th>
<th>Coefficient of Determination ($r^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sysmex KX-21N</td>
<td>12.1</td>
<td>3.0</td>
<td>Sysmex KX-21N vs</td>
<td>1.0</td>
<td>1.0</td>
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<td></td>
<td></td>
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<td>Sysmex KX-21N</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemocue 201+</td>
<td>12.0</td>
<td>2.9</td>
<td>Sysmex KX-21N vs</td>
<td>0.9959</td>
<td>0.9918</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Hemocue 201+</td>
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<tr>
<td>Mission Plus</td>
<td>12.1</td>
<td>3.1</td>
<td>Sysmex KX-21N vs</td>
<td>0.9887</td>
<td>0.9775</td>
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<td>Mission Plus</td>
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<tr>
<td>Falling Drop</td>
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<td>Falling Drop</td>
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<td></td>
</tr>
<tr>
<td>WHO Visual</td>
<td>11.9</td>
<td>3.6</td>
<td>Sysmex KX-21N vs</td>
<td>0.9423</td>
<td>0.8879</td>
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<td></td>
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<td></td>
<td>WHO Visual</td>
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</tbody>
</table>

## Cost Comparison Between 5 Hb Methods

<table>
<thead>
<tr>
<th>Method</th>
<th>Instrument Start Up Costs</th>
<th>Other Start Up Costs</th>
<th>Start up Costs</th>
<th>Reagents</th>
<th>Total Costs</th>
<th>Cost/Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sysmex KX-21N</td>
<td>$21,000</td>
<td>$250</td>
<td>$21,250</td>
<td>$250/500</td>
<td>$21,150</td>
<td>$0.50/test</td>
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<tr>
<td>Hemocue 201+</td>
<td>$500</td>
<td>$0</td>
<td>$500</td>
<td>$45/25</td>
<td>$545</td>
<td>$1.80/test</td>
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<td>Mission Plus</td>
<td>$100</td>
<td>$0</td>
<td>$100</td>
<td>$60/50</td>
<td>$160</td>
<td>$1.20/test</td>
</tr>
<tr>
<td>Falling Drop</td>
<td>$0</td>
<td>$100</td>
<td>$100</td>
<td>$10/500mL=17 tests</td>
<td>$110</td>
<td>$0.59/test</td>
</tr>
<tr>
<td>WHO Visual</td>
<td>$0</td>
<td>$0</td>
<td>$0</td>
<td>$15/150 tests</td>
<td>$15</td>
<td>$0.10/test</td>
</tr>
</tbody>
</table>

## Conclusions

- Sysmex is costly, requires stable electricity, maintenance and repairs
- Hemocue and Mission Plus are less expensive but require electricity or batteries
- Falling Drop and WHO Visual are power independent
- Falling Drop correlated well with Sysmex, Hemocue and Mission Plus
- WHO Visual was the least accurate and reproducible
- Falling Drop is a viable alternative to measure Hb w/o power

Thank you!!!!

Questions????